Effects of 13-Cis Retinoic Acid Therapy on Human Antibody Responses to Defined Protein Antigens

Neil Sidell, Michael J. Connor, Bieshia Chang, Nicholas J. Lowe, and Michael Borok

Division of Neuropathology and Brain Research Institute (NS, BC) and Division of Dermatology (MJC, NJL, MB), Jonsson Comprehensive Cancer Center, UCLA School of Medicine, Los Angeles, California, U.S.A.

We have evaluated the in vivo effects of 13-cis retinoic acid (13-cis RA) on human antibody responses to immunization with tetanus toxoid (TT) and keyhole limpet hemocyanin (KLH). Subjects with severe cystic acne were immunized with suboptimal doses (10 μg) of KLH 7 d and 3 months after starting retinoid therapy (13-cis RA, 1 mg/kg/day for 4 mo). A standard booster immunization with TT was given along with the initial KLH sensitization. A control group of acne patients received identical immunization regimens, but no 13-cis RA. Plasma retinoid levels were evaluated by reverse-phase HPLC and confirmed that blood-level concentrations of 13-cis RA and metabolites in these acne patients reached values previously demonstrated to be immunomodulatory in vitro. The retinoid had no effect on responses to TT as reflected by the characteristics of increased anti-TT IgG levels or the isotype distribution of the antibody. In contrast, the anti-KLH response was significantly enhanced in the 13-cis-RA-treated group. Whereas anti-KLH antibody was detected in only 4 of 13 control subjects after the secondary immunization, 10 of 13 retinoid-treated subjects had measurable levels of anti-KLH IgG ($p < 0.05$). Among the responders, no differences were noted in the isotype distribution of anti-KLH antibody. These results showing enhanced anti-KLH responses induced by 13-cis RA therapy represent the first demonstration in humans that in vivo administration of a retinoid can modulate antigen-specific immune responses. *J Invest Dermatol 95:597–602, 1990*

Thirteen-cis retinoic acid (isotretinoin; Accutane, Hoffmann-LaRoche, Nutley, NJ) is an effective and important treatment for severe recalcitrant cystic acne [1]. Although clinical improvement is thought to reflect a reduction in sebaceous gland size and an inhibition of sebaceous gland differentiation, immunomodulating effects of 13-cis RA have also been proposed as contributing to its mechanism of action [2]. Likewise, in clinical trials to assess the potential of 13-cis RA or other retinoids in anticancer applications, enhancement of anti-tumor responses has played an important part in the rationale for its therapeutic use [3]. Despite this background, the hypothesis that retinoids can alter immune responses is based almost entirely on animal data and in vitro human studies [4, for review]. Thus, although experiments have shown that retinoids can modulate cellular and humoral responses of human lymphocytes in vitro [5–7], there have been few reports documenting such effects in vivo. Moreover, the few in vivo studies in humans have been limited to the evaluation of non-specific immune parameters such as lymphocyte activation to mitogenic lectins or natural killer activity after retinoid supplementation [8–10]. The results have shown, at best, some marginal and transient retinoid effects that include both enhancement and suppression of natural killer activity [9] and slight changes in lymphocyte PHA responsiveness [8]. The apparent minimal effects on these parameters is supported by animal data demonstrating that retinoid-induced immune modulation in vivo requires, and is specific for, an antigenic stimulus [11,12]. Nevertheless, at the present time there have been no reported studies to determine if retinoid therapy can affect human antigen-specific responses.

The first evidence that retinoids can alter human antibody production was the demonstration that retinoic acid (RA) can enhance responses of normal tonsil B cells to heterologous antigens in vitro [6]. Other studies have shown enhancement of in vitro human B-cell responses by RA using lymphocytes from different compartments [13] or disease states [14]. The present work was designed to determine whether 13-cis RA can modulate specific human responses in vivo. To do so, we have evaluated the ability of this retinoid to modulate humoral responses in acne patients to tetanus toxoid (TT) and keyhole limpet hemocyanin (KLH) as defined-protein antigens. Our results have established that 13-cis RA therapy can enhance specific immune reactions. However, the findings suggest that this agent has only limited immunomodulating activity, which may be dependent on the type and degree of immunologic challenge.

MATERIALS AND METHODS

Patient Treatment and Immunization Twenty-six subjects were selected from student volunteers being evaluated for treatment of severe recalcitrant cystic acne by the Division of Dermatology, Department of Medicine, UCLA School of Medicine. Informed consent for this study was obtained from each individual according to the guidelines established by the Human Subjects Protection Committee, UCLA School of Medicine. Subjects were determined to be in good health (other than their dermatologic condition) after a medical history, physical examination, and standard laboratory

Manuscript received April 12, 1990; accepted for publication June 28, 1990.

Supported by National Institutes of Health grants CA30515, CA43503, NS and CA47758, MJC.

Reprint requests to: Dr. Neil Sidell, Department of Pathology, 18-170 Center for Health Sciences, UCLA School of Medicine, Los Angeles, CA 90024.

Abbreviations:
13-cis RA: 13-cis retinoic acid
ELISA: enzyme-linked immunosorbent assay
IgG-KLH: anti-KLH IgG antibody
IgG-Tet: anti-tetanus toxoid IgG antibody
KLH: keyhole limpet hemocyanin
TT: tetanus toxoid

0022-202X/90/$03.50

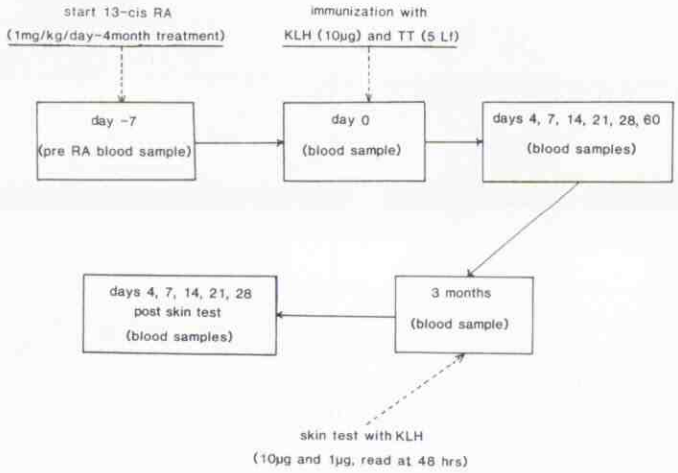

Figure 1. Immunization and blood sampling protocol for 13-cis RA-treated subjects. Controls had the identical immunization and blood-drawing regimen without the retinoid.

determinations. None had a history of clinical or laboratory findings consistent with impaired renal, hepatic, or immunologic functions, and none were receiving excessive vitamin A intake or had a history of sensitivity to vitamin A or any of its derivatives. Standard laboratory tests to evaluate hematologic, liver, and renal function were performed periodically during treatment. Subjects in the treatment group (10 men, three women; mean age ± SD 22.0 ± 2.7 years) received 13-cis retinoic acid (Accutane) at 1 mg/kg/d twice daily for 4 months. The immunizing protocol with keyhole limpet hemocyanin (KLH) and tetanus toxoid (TT), and the schedule of 14 blood-draws/patient over the 4 mo is shown in Fig 1. The control group (nine men, four women; mean age ± SD 24.0 ± 5.3 years) consisted of acne patients whose disease, immunization, and blood-drawing regimens were identical to the treatment group, but for the use of 13-cis RA. For each blood sampling, serum (for measuring antibody levels) and plasma (for measuring retinoid levels) were obtained and stored in multiple frozen aliquots.

The KLH (Pacific Biomarine Supply Co., Venice, CA) used for immunization was prepared according to the method of Campbell et al [15], then dialyzed against isotonic saline, sterilized by filtration, and stored in aliquots of 100 μg/ml under liquid nitrogen. Absence of contaminating pyrogens were confirmed by a lack of response in four rabbits, each injected subcutaneously with 5 mg KLH. Subjects were immunized intradermally with 10 μg KLH in 0.1 ml of saline on the volar aspect of the forearm and then, 3 mo after this primary immunization, were skin tested with 1 μg and 10 μg KLH that also served as the secondary immunization (Fig 1). Ten μg KLH has been reported to result in submaximal responses in humans [16,17]. Our rationale for choosing a suboptimal immunizing dose was based on animal data and our in vitro human studies that indicated that maximal retinoid-induced immune enhancement occurs with suboptimal sensitization to antigen [6,11,18]. A booster intramuscular immunization with 5 Lf of soluble TT (Wyeth Laboratories Inc., Marietta, PA) was given along with the initial KLH sensitization.

Plasma Retinoid Concentrations Because of the sensitivity of retinoids to light, blood samples used to determine plasma retinoids were collected into aluminum foil-covered vacutainer tubes and immediately processed under minimal lighting. Plasma samples were stored frozen at −20°C until assayed. Samples were processed by adding 0.05 ml plasma to 0.2 ml analytic grade methanol containing the antioxidant butylated hydroxytoluene (0.1 mg/ml) and

Table I. Plasma Retinoid Levels in 13-cis RA-Treated Subjects[a]

Patient	Months of Treatment	Retinoid Concentration (μmol/l Plasma)				
		13-cis RA	All-Trans RA	Retinol	4-Oxo-	4-Hydroxy-
A	1	1.80	0.13	2.70	8.22	2.72
	2	1.33	0.09	2.00	8.21	4.46
B	1	1.19	0.03	1.90	4.89	2.27
	2	2.13	0.01	1.95	4.85	2.11
C	1	1.64	0.04	1.21	4.28	2.75
	2	3.33	0.01	1.15	7.18	2.90
D	1	1.23	0.08	2.50	7.18	2.81
	2	1.36	0.01	1.87	6.33	2.83
E	1	1.79	0.08	3.03	3.22	2.14
	2	1.83	0.20	2.40	4.09	2.24
F	1	1.60	0.05	2.18	7.62	3.17
	2	1.25	0.05	1.96	6.21	2.59
G	1	2.60	0.01	1.50	9.33	3.27
	2	1.72	0.02	1.94	7.67	2.89
H	1	1.16	0.14	1.53	7.43	2.95
	2	1.06	0.02	1.97	6.07	2.79
I	1	1.57	0.03	2.16	3.52	2.00
	2	0.80	ND[b]	1.93	3.98	3.23
J	1	1.32	ND	2.63	7.40	2.13
	2	2.23	0.04	2.40	5.70	2.45
K	1	0.92	0.07	2.26	3.16	2.69
	2	1.58	0.09	2.22	5.56	2.27
L	1	1.08	0.03	1.31	8.24	5.90
	2	1.76	0.10	1.55	5.62	2.14
M	1	2.51	0.26	1.17	8.76	2.84
	2	1.72	0.08	1.46	9.27	3.53
Mean ± SD	(n = 26)	1.64 ± .58	0.07 ± .06	1.95 ± .49	6.34 ± 1.93	2.88 ± .82
Controls	(n = 4)	ND	ND	1.76 ± .43	ND	ND

[a] 1 mg/kg/d.
[b] Not detected.

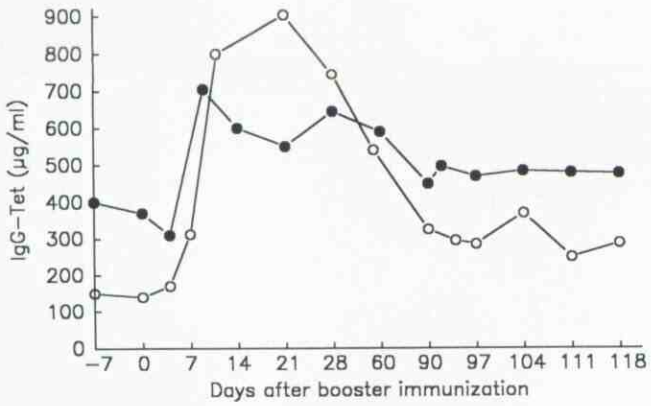

Figure 2. IgG-Tet levels in the serum of two 13-cis-RA–treated subjects following TT booster immunization. *Subject A* (○) had not had a previous booster for > 10 years, whereas *subject D* (●) had had a booster 9 mo before the present one. These profiles are representative for both treatment and control subjects and illustrate that, in both groups, increases in IgG-Tet were generally greater in individuals that had not had a previous TT booster within the last 3 years.

200 ng TTNPB ethyl sulfone (RO 15-1570, Hoffmann La-Roche Inc.) as the internal standard. Retinoid concentrations were determined by reverse-phase HPLC as previously reported [19], using 22 parts 0.01 M potassium acetate and 78 parts methanol (made to pH 6.6 with acetic acid) as solvent.

Enzyme-Linked Immunosorbent Assays (ELISA) for Anti-Tetanus Toxoid IgG Antibody (IgG-Tet) Levels The solid-phase quantitative ELISA for IgG-Tet was adapted from the Tet-radioimmunoassay of Stevens and Saxon [20] by substituting alkaline phosphatase-coupled antibody for radioiodinated antibody in the developing antibody step [21]. Results are expressed as μg IgG-Tet per ml of patient serum.

To assess IgG isotype responses to TT, a four-layer ELISA was developed. As for the IgG-Tet ELISA, microtiter plates were initially coated with TT, washed, and then incubated with serum samples. However, for these determinations, the second antibody was anti-isotype–specific mouse monoclonal (Caltag Laboratories, South San Francisco, CA), followed by alkaline phosphatase-coupled goat anti-mouse IgG (Caltag Laboratories). All the second antibodies were of IgG_1, κ class. Results are expressed as μg of bound-mouse IgG per ml of serum, by reference to standard curves obtained by coating wells with goat anti-mouse IgG followed by affinity-purified mouse IgG (Caltag Laboratories). By analyzing sequential samples from each patient, these results could be used for monitoring subclass-specific anti-TT responses as well as for comparing IgG-Tet isotype prevalence between the treatment and control groups.

"Enhanced" ELISA for Anti-KLH Antibody Production To optimize detection of anti-KLH antibody in patient serum resulting from the small KLH-immunizing dosage (10 μg), we developed an "enhanced" ELISA as originally described by Self et al [22]. This assay was performed in KLH-coated microtiter plates using enhancing reagents now available commercially from GIBCO/BRL (Gaithersburg, MD). Reagents for color development were prepared and used according to manufacturers instructions (GIBCO/BRL). Results are expressed as μg IgG-KLH per ml of patient serum obtained by subtracting background values determined from pre-treatment (pre–KLH-sensitization) samples.

Anti-KLH IgG (IgG-KLH) isotype responses were assessed from patient samples by a four-layer "enhanced" ELISA. This assay is essentially identical in methodology and theory to the four-layer ELISA system used for determining IgG-Tet subclass responses with the additional modifications employed for the amplified developing scheme. Results are expressed as μg of bound-mouse IgG per ml of patient serum by reference to standards as described for the IgG-Tet isotype ELISA.

RESULTS

Retinoid Levels Plasma from 13-cis RA-treated subjects routinely showed the presence of 13-cis RA, two major polar metabolites that coeluted with 4-hydroxy-13-cis RA and 4-oxo-13-cis RA, and all-trans RA as a minor peak. Table I shows the retinoid levels in the 13 retinoid-treated subjects and four randomly selected controls from samples taken one and two months after the onset of treatment. As seen, retinoid levels in the two samples from each treatment subject were in generally good agreement and indicated an average (± SD) 13-cis RA blood level concentration of 1.64 ± 0.58 μM. The average concentrations (± SD) of all-trans RA, 4-oxo-13-cis RA, and 4-hydroxy-13-cis RA (the most prevalent 13-cis RA metabolites) were 0.07 ± .06, 6.34 ± 1.93, and 2.88 ± .82 μM, respectively. None of these retinoids were detected in the pretreatment samples or in those obtained from the controls. Retinol was present in samples from all subjects, and the level was not affected by the 13-cis RA administration (mean treated vs control; 1.95 ± .49 vs 1.76 ± .43 μM).

Anti-TT Responses Serum IgG-Tet levels were measured for 4 months following booster intramuscular immunization with 5 Lf of soluble tetanus toxoid. Figure 2 shows representative profiles of IgG-Tet levels in the serum of two 13-cis-RA–treated subjects. No anti-TT IgM was detected at any time in the serum samples. Responses in both groups generally fit the reported typical characteristics [23]: peak IgG-Tet levels (up to > 1000 μg/ml) usually occurred between 7 and 21 d post immunization and slowly decreased to about one-half maximum after the 4-mo period; relative increases in antibody levels were generally greater in individuals that had not had a previous tetanus booster within the last 3 y. A summary of pertinent IgG-Tet response parameters for the two groups of subjects is shown in Table II. As previously described, large variations were seen between individuals, probably reflecting the length of time since their last TT booster immunization [23,24]. No signifi-

Table II. IgG-Tet Responses[a]

Group	Pre-booster Level (μg/ml)	Time to Peak (days)	Peak Level (μg/ml)	Increase (μg/ml)	Relative Increase	4-Mo Level (μg/ml)
Control	184 ± 63	17 ± 4	654 ± 94	469 ± 82	7.8 ± 2.0	413 ± 62
13-cis RA	148 ± 43	21 ± 4	709 ± 165	561 ± 163	6.9 ± 2.3	359 ± 73

[a] Measured by ELISA in patient sera after booster immunization with 5 Lf of soluble tetanus toxoid. Results are expressed as mean ± SEM of the indicated response parameter for the 13 subjects in each group. No IgM-Tet was detected in any serum sample.

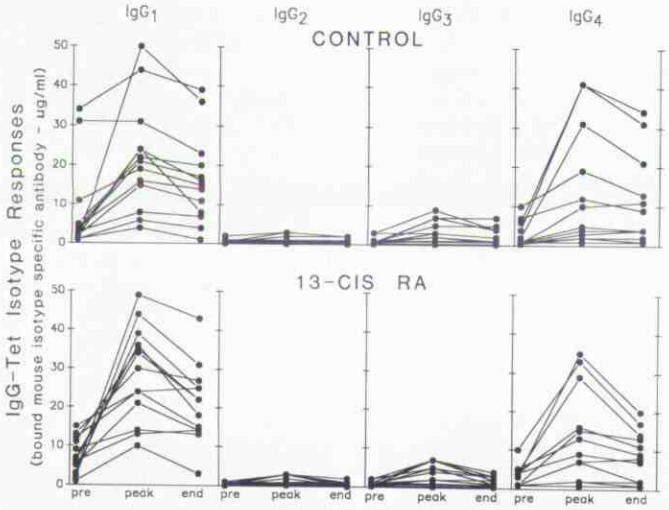

Figure 3. IgG-Tet isotype responses of control and 13-cis RA-treated subjects. Anti-TT IgG subclass levels were assessed by a four-layer isotype-specific ELISA in serum samples drawn from subjects before their tetanus booster *(pre)*, at the time of peak IgG-Tet levels *(peak)*, and at the end of their 4-mo blood-drawing protocol *(end)*. Results are expressed as μg of bound-mouse isotype-specific antibody/ml of patient serum as described in *Materials and Methods*. There was no significant difference between the two groups in their IgG subclass responses to TT.

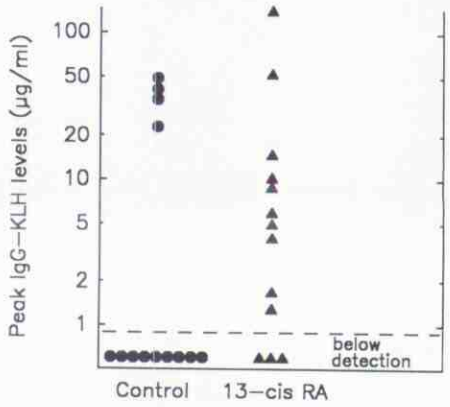

Figure 4. Peak IgG-KLH levels following KLH skin test (secondary immunization). There was a significant difference between the two groups in the number of subjects showing detectable levels of anti-KLH antibody ($p < 0.05$ by Fisher's exact test). No anti-KLH IgM was detected. Antibody levels were determined using an "enhanced" KLH ELISA as described in *Materials and Methods*.

cant differences were found in the characteristics of the serum anti-TT antibody responses of the treatment group compared with controls.

Sera were also assayed pre-TT immunization, at the time of peak IgG-Tet levels for each subject as determined above, and at the end of the 4-mo treatment protocol to evaluate whether the retinoid treatment had any effect on IgG-subclass responses to TT. Figure 3 indicates that anti-TT responses in both groups were predominantly of the IgG_1 isotype, with all retinoid-treated subjects and all but one control showing marked increases in anti-TT IgG_1 following the booster immunization. A large variability was observed in the amount of IgG_3 and IgG_4, with IgG_3 showing lower responses of the two. In all cases, very little IgG_2 could be detected. No significant differences were found between the treatment and control groups in their IgG subclass responses to TT.

Anti-KLH Responses We tested for anti-KLH Ig in serum samples taken in the 3-mo period following the primary KLH sensitization and in the month following the secondary skin-test immunization. Neither anti-KLH IgM nor IgG were detected in any sample taken between the initial KLH immunization and before the 3-mo KLH skin test. Following the skin test, anti-KLH IgG, but not IgM, was detected in some subjects' sera (Fig 4). There was a significant difference between the control and treatment groups: antibody (1–142 μg/ml) was detected in 10 of 13 13-cis RA-treated samples but in only 4 of 13 control samples ($p < 0.05$ by Fisher's exact test). A positive skin-test reaction was observed in only two subjects, both in the 13-cis RA treatment group. As shown in representative profiles (Fig 5), serum levels of IgG-KLH reached maximum values 7–21 d after the secondary immunization.

IgG subclass responses to KLH were assessed in the four control subjects that showed detectable anti-KLH responses and in the four 13-cis RA-treated subjects showing the highest IgG-KLH levels following secondary stimulation with KLH. As seen in Table III, anti-KLH IgG_1 was generally the predominant subclass detected in both groups, with IgG_2 responses also being apparent. Small quantities of anti-KLH IgG_3 were present in about half the sera tested, whereas responses of the IgG_4 isotype were not detected except in one case (control). There were no apparent differences in relative anti-KLH IgG subclass responses between the subjects tested in the two groups.

DISCUSSION

In this study, we have assessed the ability of systemic 13-cis RA to modulate specific antibody responses to TT booster immunization and primary and secondary immunization with KLH in acne patients. Plasma retinoid levels were evaluated for all retinoid-treated subjects by reverse-phase HPLC and indicated 13-cis RA concentrations in the μM range, with the 4-oxo and 4-hydroxy metabolites attaining blood level concentrations even higher than the parent compound. These results showed compliance by all of the subjects and indicated that plasma concentrations of 13-cis and all-trans RA reached on average 1.6×10^{-6} and 7×10^{-8} M, respectively, values previously demonstrated to be immunomodulatory in vitro [5,6]. Our determination of concentrations of 13-cis RA and its metabolites agreed with those obtained in previous studies of acne patients on long-term 13-cis RA therapy [25,26]. Retinol levels were not affected by the 13-cis RA administration. This finding does not support the contention that retinoic acid may interfere

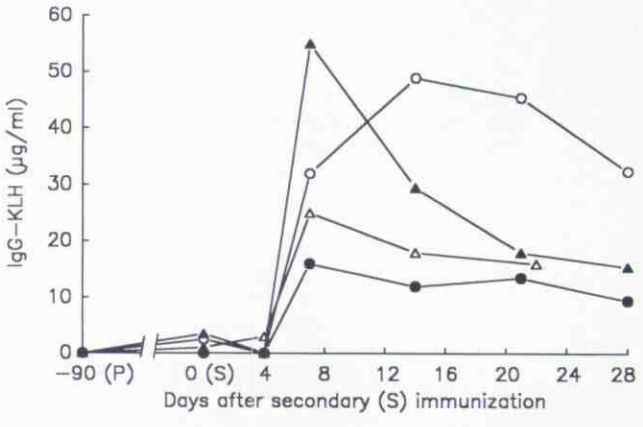

Figure 5. Concentration of IgG-KLH in the serum of two control (○,△) and two 13-cis-RA-treated subjects (●,▲) following primary *(P)* and secondary *(S)* immunization with 10 μg KLH. These profiles are representative for both groups and indicate that no anti-KLH antibody was detected in serum samples taken between the primary sensitization with this suboptimal dose of KLH and the 3-month KLH skin test (secondary immunization).

Table III. IgG-KLH Isotype Levels[a]

	IgG_1[b]	IgG_2	IgG_3	IgG_4
Accutane Patients				
E	4.4	3.3	0.3	<.1
H	2.4	<.1	0.2	<.1
J	0.1	0.7	<.1	<.1
K	1.1	0.4	<.1	<.1
Control Patients				
Q	1.1	1.0	<.1	<.1
U	0.4	0.3	<.1	<.1
W	2.2	0.2	0.9	<.1
X	1.6	2.0	0.4	0.4

[a] Measured by a four-layer enhanced isotype-specific ELISA as described in *Materials and Methods*.

[b] Assessed in serum samples drawn from patients at the time of peak IgG-KLH levels. Results are expressed as μg of bound-mouse isotype-specific antibody/ml of patient serum.

with absorption or mobilization of retinol, as suggested by Keilson et al [27].

Our results establish that 13-cis RA can enhance human immune responses in vivo. Retinoid-treated subjects showed a significantly higher incidence of detectable levels of specific antibody production following immunization with 10 μg KLH than did control subjects. Previous work with KLH in humans have demonstrated vigorous primary and secondary responses after sensitization to much higher doses than those used in our protocol [16,28]. Our rationale for choosing a suboptimal immunizing dosage was based on animal and in vitro human studies indicating that maximal retinoid-induced immune enhancement occurs during suboptimal sensitization to antigen [11,18]. As such, our inability to detect anti-KLH antibody in sera taken after the primary but before the secondary immunization has confirmed that the subjects were, indeed, immunized with a very submaximal dose of KLH. Fortuitously, this fact seems to have highlighted the immune-enhancing effect of the retinoid therapy, as reflected by the significant increase in discernable IgG-KLH in the treatment group after the secondary immunization. From the present data, we cannot distinguish whether the 13-cis RA was acting on the primary and/or secondary phase of the sensitization process, or which cell type(s) was being affected. With regard to this latter point, in vitro studies have shown that RA can directly affect the immune functions of both B- and T-cell populations [5,6].

When assessing IgG-KLH subclass responses, we found IgG_1 to be the predominant isotype in most subjects, with no apparent difference between treatment groups. Unlike the anti-TT response, where very little IgG_2 could be measured, IgG_2 was seen to be the second-most prevalent anti-KLH isotype after IgG_1, with almost no anti-KLH IgG_4. These differences in subclass levels between TT and KLH may reflect the highly immunogenic nature of carbohydrate moieties on the latter [29]. IgG_2 has been shown to be a major component of human antibody responses to carbohydrate antigens [30].

Our results did not indicate any differences between the groups in their responses to booster TT immunization as reflected by a variety of parameters: 1) regardless of treatment, peak IgG-Tet usually occurred between 7 and 21 d post-immunization and slowly decreased to about one-half maximum after the 4-mo period; 2) relative increases in IgG-Tet were similar in both groups; and 3) anti-TT antibody responses were predominantly of the IgG_1 subclass, with little IgG_2, IgG_3, and no IgM-Tet being detected. This anti-TT isotype distribution is characteristic of a booster response to TT [31,32] and the present results indicated no shift in this distribution caused by the retinoid therapy. The fact that 13-cis RA did not influence the kinetics and subclass characteristics of the TT response indicates that this retinoid does not profoundly alter the complex network of regulatory events that occur during a strong booster response. However, the optimal reimmunization protocol used in this study would not likely reveal the ability of 13-cis RA to enhance anti-TT responses; rather this would probably require reimmunization with much lower TT doses. Alternatively, enhancement by 13-cis RA may be dependent upon its presence during primary sensitization, as was the case with KLH, whereas the retinoid may have little effect when its use is limited to recall (booster) reactions, no matter what the reimmunization dose.

Finally, our data support the contention that stimulation, rather than suppression, of acne-associated immune responses occurs during 13-cis RA therapy [2]. Thus, whereas 13-cis RA has no direct antibacterial effects, the number of *Propionibacteria* and *Micrococci* are significantly reduced in treated patients [2,33]. In light of the present results, we hypothesize an immunologic contribution to this reduction by the ability of the retinoid to increase relevant responses to weakly immunogenic bacterial epitopes. Whether or not such action might significantly contribute to the clinical efficacy of 13-cis RA is presently unknown. Future studies will address these questions, as well as further delineate the conditions and mechanism(s) by which retinoids can function as immune modulators in different clinical settings.

We wish to thank Luiza Vartavarian for excellent technical assistance. The 13-cis RA (Accutane) was generously contributed by Hoffmann-LaRoche, Inc., Nutley, NJ.

REFERENCES

1. Peck GL, Olsen TG, Yoder FW, Strauss JS, Downing DT, Pandya M, Butkus D, Arnaud-Battandier J: Prolonged remissions of cystic and conglobate acne with 13-cis retinoic acid. N Engl J Med 300:329–333, 1979
2. Plewig G, Nikolowski J, Wolff HH: Action of isotretinoin in acne rosacea and gram-negative folliculitis. J Am Acad Dermatol 6:766–785, 1982
3. Lotan R: Immunomodulating effects of retinoids. J Nutr Growth Cancer 3:57–65, 1986
4. Dennert G: Retinoids and the immune system: immunopotentiation by vitamin A. In: Sporn MB, Roberts AA, Goodman DS (eds.). The Retinoids. Academic Press, New York, 1984 pp 373–390
5. Sidell N, Rieber P, Golub SH: Immunological aspects of retinoids in humans. I Analysis of retinoic acid enhancement of thymocyte responses to PHA. Cell Immunol 87:118–125, 1984
6. Sidell N, Famatiga E, Golub SH: Immunological aspects of retinoids in humans II. Retinoic acid enhances induction of hemolytic plaque-forming cells. Cell Immunol 88:374–381, 1984
7. Skinnider LF, Giesbrecht K: Inhibition of phorbol myristate acetate and phytohemagglutinin stimulation of human lymphocytes by retinol. Cancer Res 39:3332–3334, 1979
8. Soppi AM, Soppi E, Eskola J, Jansen CT: Cell-mediated immunity in Darier's disease: effect of systemic retinoid therapy. Br J Dermatol 106:141–152, 1982
9. McKerrow KJ, Mackie RM, Lesko MJ, Pearson C: The effect of oral retinoid therapy on the normal human immune system. Br J Dermatol 119:313–320, 1988
10. Holland DB, Gowland G, Cunliffe WJ: Inflammatory responses in acne patients treated with 13-cis-retinoic acid (isotretinoin). Br J Dermatol 110:343–345, 1984
11. Dennert G, Lotan R: Effects of retinoic acid on the immune system: stimulation of T killer cell induction. Eur J Immunol 8:23–29, 1978
12. Soppi E, Lehtonen OP: Selective effect of different retinoids on the primary antibody response in normal chickens. Immunopharmacol 8:91–96, 1984
13. Dillehay DL, Cornay WJ, Walia AS, Lamon EW: Effects of retinoids on human thymus-dependent and thymus-independent mitogenesis. Clin Immunol Immunopath 50:100–108, 1989

14. Sherr E, Adelman DC, Saxon A, Gilly M, Wall R, Sidell N: Retinoic acid induces the differentiation of B cell hybridomas from patients with common variable immunodeficiency. J Exp Med 168:55–71, 1988
15. Canpbell DH, Garvey JS, Cremer NE, Sussadorf DH: Methods in Immunology, Benjamin, New York, 1964, pp 69–70
16. Curtis JE, Hersh EM, Harris JE, McBride C, Freireich EJ: The human primary immune response to Keyhole limpet haemocyanin: interrelationships of delayed hypersensitivity, antibody response, and in vitro blast transformation. Clin Exp Immunol 6:473–491, 1970
17. Curtis JE, Hersh EM, Butler WT, Rossen RD: Antigen dose in the human immune response: dose-response relationships in the human immune response to Keyhole limpet hemocyanin. J Lab Clin Med 78:61–69, 1971
18. Sidell N, Famatiga E, Golub SH: Augmentation of human thymocyte proliferative responses by retinoic acid. Exp Cell Biol 49:239–245, 1981
19. Connor MJ, Smit MH: Retinoic acid formation from retinol in the skin. Biochem Pharmacol 36:919–924, 1987
20. Stevens RH, Saxon A: Immunoregulation in humans: control of anti-tetanus toxoid antibody production after booster immunization. J Clin Invest 62:1154–1160, 1978
21. Stevens R, Oliver M, Brogan M, Heiserodt J, Targan S: Defective generation of tetanus-specific antibody-producing B cells after in vivo immunization of Crohn's disease and ulcerative colitis patients. Gastroenterology 88:1860–1866, 1985
22. Self CH: Enzyme amplification — a general method applied to provide an immunoassisted assay for placental alkaline phosphatase. J Immunol Method 76:389–393, 1985
23. Stevens RH, Saxon A: Reduced in vitro production of anti-tetanus toxoid antibody after repeated in vivo immunization with tetanus toxoid. J Immunol 122:592–598, 1979
24. Kodo H, Gale RP, Saxon A: Antibody synthesis by bone marrow cells in vitro following primary and booster tetanus toxoid immunizations in humans. J Clin Invest 73:1377–1384, 1984
25. Brazzell RK, Vane FM, Ehmann CW, Colburn WA: Pharmacokinetics of isotretinoin during repetitive dosing to patients. Eur J Clin Pharmacol 24:695–702, 1983
26. Kerr IG, Lippman ME, Jenkins J, Myers CE: Pharmacology of 13-cis-retinoic acid in humans. Cancer Res 42:2069–2073, 1982
27. Keilson B, Underwood BA, Loerch JD: Effects of retinoic acid on the mobilization of vitamin A from the liver of rats. J Nutr 109:787–795, 1979
28. Curtis JE, Hersh EM: The human secondary immune response to Keyhole limpet haemocyanin. Clin Exp Immunol 10:171–177, 1972
29. Grzych JM, Dissous C, Capron M, Torres S, Lambert PH, Capron A: Schistosoma Mansoni shares a protective carbohydrate epitope with keyhole limpet hemocyanin. J Exp Med 165:865–878, 1987
30. Yount WJ, Dorner MM, Kunkel HG, Kabat EA: Studies on human antibodies. VI Selective variations in subgroup composition and genetic markers. J Exp Med 127:663–646, 1968
31. Seppala IJT, Routonen N, Sarnesto A, Mathla PA, Makela O: The percentage of six immunoglobulin isotypes in human antibodies to tetanus toxoid: standardization of isotype-specific second antibodies in solid phase assay. Eur J Immunol 14:868–875, 1984
32. Stevens R, Dichek D, Keld B, Heiner D: IgG_1 is the predominant subclass of in vivo- and in vitro-produced antitetanus toxoid antibodies and also serves as the membrane IgG molecule for delivering inhibitory signals to anti-tetanus toxoid antibody-producing B cells. J Clin Immunol 3:65–69, 1983
33. Weissmann A, Wagner A, Plewig G: Reduction of bacterial skin flora during treatment with 13-cis-retinoic acid. Arch Dermatol Res 270:179–183, 1981